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ENVIRONMENTAL RELATIONS OF MODIFICATION COMPOSITIONS OF CERTAIN CARBONATE SECRETING MARINE INVERTEBRATES

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Studies by numerous investigators, both incidental and direct, on the modification composition of skeletal carbonate in recent marine organisms have established that the stable modification calcite and the metastable aragonite constitute the commonly secreted constituents, singly or in combination.[1-6] Mostly going hand-in-hand with studies of skeletal micro-architecture, the purpose of the investigations has been to determine the distributional representation of the two modifications through the spectrum of carbonate synthesis with reference to the successive levels of organization. Undertaken for obvious reasons along taxonomic lines, random selection of species secured from biologic collections served in general for the analyses. The locations of specimen derivation and relation to ecologic aspects were in almost all instances of no concern, the compositional determinations serving primarily to add to the taxonomic diagnosis of the species examined. Data accumulated over a period of time on the mineralogic composition of random selected species within most classes from the family level up, in some cases as high as the phyllum level, as in the echinodermata, showed mostly identical modification composition. From this the concept evolved that with the exception of the two molluscan classes, the gastropods and pelecypods, comparatively uniform modification synthesis is the rule, the skeletons within taxonomically deformed limits consisting either entirely of calcite or aragonite. In the two molluscan classes, however, considerable complexity is involved. Calcite and aragonite occur there not only singly in skeletons as in the other classes, but both minerals may partake in the composition of single shells. In such instances the two modifications form micro-architecturally separate elements. As far as the distribution is concerned, the modification composition was found to differ in certain families and in some instances noted between species within a genus.[3] It is perhaps for this reason that these two molluscan classes have been investigated more thoroughly from the statistical point of view than any others. By now there exist diagnoses of the modification composition for all known carbonate secreting groups, though to judge from the literature controversies are in evidence in a few instances. They involve the entire phyllum bryozoa,[2, 3, 7, 8, 9] the serpulidae[2, 8, 9, 10, 11] among the polychaete worms, and recently certain molluscan species.[3, 6] Analysis of the reasons for these particular uncertainties show that, frequently, later determinations of random selected representatives of these groups differed from those made earlier, leading to questioning of the accuracy of determinations of one or another investigator. This is perhaps best illustrated by Stolkowski's[6] recent disagreement as to the composi-

tion of an individual of the same species of *Haliotis* determined by him to consist entirely of aragonite, as contrasted to the earlier diagnosis by Bøggild[2] stating that a volumetrically small calcitic layer is intercalated between the well-developed aragonitic ones. The basic philosophy involved, though not stated in these words, seems to pertain to the generally held concept that the modification synthesis products on the species level, and in most categories for the entire groups diagnosed, are constant. Thus, the mineralogic composition was inferred to be rigidly, that is genetically fixed. This perhaps explains the reason why the locations of respective sample derivations remained beyond consideration in the evaluation of the differences in modification determinations. This very aspect would involve the possibility of ecologic controls. This question was actually considered by Bøggild.[3]

TABLE I

SERPULIDAE WORM TUBES

LOCATION	LATITUDE	T. °C., MEAN AND RANGE	ARAGONITE, %
Malmøya, Oslo Fiord, Norway (3)	59°40′ N.	10.4 0.22–22.03	62
St. Paul Is., Pribilofs (1)	57°20′ N.	4.0 0.24–9.94	0
Southwestern coast Amchitka, Aleutians (2)	51°37′ N.	5.0 2.2°–8.7	0, 0, 0, 0, 0, 0, 0, 15, 22
Gloucester Point, Va. (6)	37°15′ N.	22.9 14.7–27.3	59, 64
(8)		24.5 20.0–27.3	53, 56
(9)		25.1 19.9–28.1	74, 84
Okinoshima, Honshu, Japan (4)	35° N.	18 12.1–24.2	28, 39, 41, 43, 50, 51
La Jolla, Calif. (5)	32°50′ N.	17 14.0–20.77	18, 29, 53, 54
Bermuda: (7) inshore waters	30°20′ N.	23 16–30	58, 63, 66, 68, 70, 76, 76, 77, 80, 80, 85, 86, 86, 89, 96, 96
Mangrove Lake			98, 98
Ngerikui, Passage of Koror Island, Palau (10)	7°19′ N.	28.0 27.5–29.5	90, 96, 96

His conclusion however, based on admittedly limited data, was that ecologic variables were not effective. Since then, no further consideration has been given to the possibility of ecologic relations to the modification composition in carbonate skeletons.

It is the purpose of the following presentation to show that within certain classes the mineralogy of skeletal materials is environment-dependent. The survey on which this study is based pertains principally to the analysis of one of the groups open to question in the literature, the serpulidae, and considers examples similarly found by the writer among the gastropods and pelecypods only as far as they contribute to the elucidation of the problem.

Modification compositions in the study were determined on powdered smear slide preparations by means of a Phillips x-ray spectrometer with attached Brown Recorder. Whenever both calcite and aragonite were found to be present, their relative amounts were found using a calibration curve derived from synthetic mixtures and established by K. E. Chave and further improved by L. Silver and H. Fahy. The accuracy of the measurements is $\pm 10\%$. The uncertainty in the measurements of the amount of mineral decreases if the ratio of the two forms is less than 1/10. Because of the small size of most serpulidae worm tubes examined, determinations are largely based on composites of a number of them to assure large enough samples. In view of the composite nature of most of these samples and the limited accuracy of the mineralogical determinations where both modifications are involved, the significance of the serpulidae data must rest entirely on trends rather than the percentage determinations *per se*.

To assure proper comparison between modification composition and ecologic conditions, in particular environmental temperatures and salinities, the survey is based on recently-collected specimen series either from localities where the yearly climatic amplitude and salinity ranges of the inhabiting waters were available, or at least from locations nearby to hydrographic stations where there were no indications of marked micro-environmental deviations. In view of the survey nature of the investigation the samples for the study were selected from widely distant locations extending from high to low latitudes. Specific identifications for most of the forms employed in the study are lacking.

Table 1 shows the modification composition of the serpulidae worm tubes analyzed, arranged in the order of their latitudinal derivations from low to high latitudes. The modification compositions determined range from 100% calcite through varying percentages of calcite and aragonite to 98% aragonite. In general the modification composition of the serpulidae worm tubes is shown not only to vary from one geographic location to another, but also within local populations. As illustrated by the Bermuda samples, which encompass the largest number of analyses from any single locality, the range in compositional variation extends far beyond the limits of error of the method involved. Only the Aleutian samples constitute a possible exception. Two of the initial runs out of a total of seven showed small amounts of aragonite. Since subsequently run samples gave 100% calcite throughout, it is suspected that we are dealing in these early identifications with admixtures of small amounts of aragonitic shell substrates from which the worm tubes had been removed. If so, the Amchitka serpulidae would constitute a decided exception to constancy in modification composition. Analysis of the overall variability shows that even when viewed on as general a basis as latitudinal distribution that entirely calcitic tubes are confined to the high latitudes, the subarctic, while mixtures with more than 85% aragonite are derivatives from the subtropics and tropics. The modification compositional ranges for the samples of each locality were therefore plotted against yearly mean temperatures of their inhabiting waters. The results (Fig. 1), show that at least partial temperature-controlled modification synthesis is involved. Viewed within the framework of the gross temperature effect on the modification synthesis products for this group, the observed variability shown to extend down to the population level of single localities is not only compatible but should exist once the consideration is extended to the ranges

of the yearly amplitude of the inhabiting waters. If the synthesis of the modification types or relative amounts in the tube formations are in this group in some way and within certain limits temperature dependant, the mean averages of individuals or composite samples should vary according to the time interval over which the tubes formed, the yearly temperature range, and the cumulative response in volume secretion per temperature integral thereof. Hence in waters with a wide yearly amplitude, such as at Bermuda, where temperatures range from 16 to 30°C. and where we have evidence that serpulidae worms grow, as well as spawn, in winter and summer, variations in modification composition over the range exhibited are to be expected. The Bermuda sample series from the inshore waters alone is most suitable to explore further this inference. This series consists of the largest number of

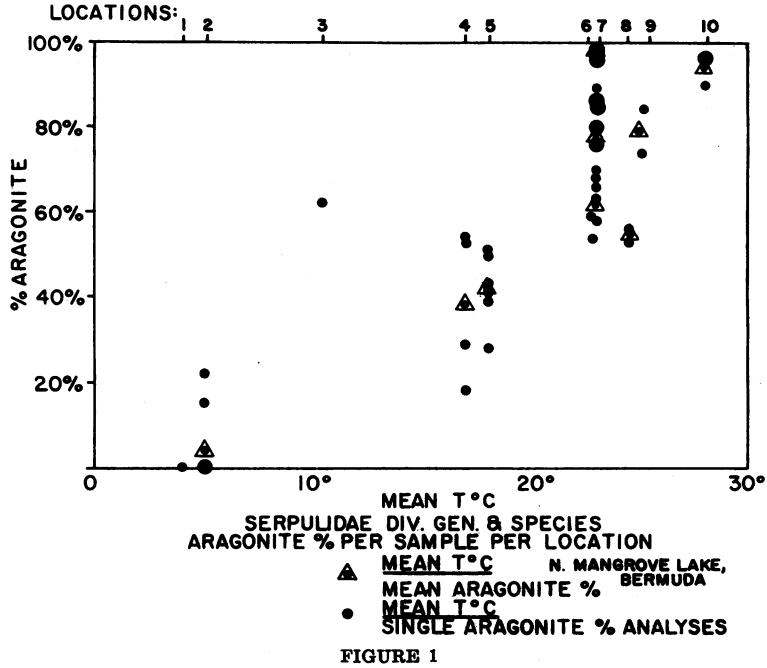

FIGURE 1

Closed dots represent single aragonite analyses; circled dots represent the mean value for two or more aragonite analyses per species. Dots surrounded by triangles represent the average of all the analyses for all species considered in a single location. The triangular point located on the graph at 60% aragonite and 23°C should be deleted.

samples analyzed, hence statistically gives greater assurance of covering more accurately the range of variations in aragonite percentages involved.

There is another aspect which seems equally critical for the selection. If the temperature environment does enter into the controls of skeletal modification synthesis, other environmental factors might also be involved, though perhaps to a lesser degree, and be responsible for some of the more marked deviations from the growth trend, noticeable in figure 1, which cannot be attributed to error of the determination method. A factor to be considered is salinity since it is known to affect skeletal morphology. In the Bermuda inshore waters salinity is virtually

constant the year around, at the most fluctuating 1 per mille from the norm of 35 per mille, and hence can be eliminated from the consideration. Although the variables which may enter into the analysis of the Bermuda population samples are thus reduced, the information available for examining the relations of the aragonite percentages to temperatures of synthesis is far from satisfactory. In only one case, which involves one of the two lowest aragonite percentages determined, is there comparatively more precise information on the growth period available. The sample, analyzing 63% aragonite, represents a composite of several small tubes grown during the intermolt period on the carapace of an individual of *Panulirus argus*, kept under observation in a tank at the Biological Station, with daily temperature records. Although there is no assurance that the beginning of growth of the serpulidae worm tubes coincided with that of the intermolt period, the fact that the temperature range covered by the latter falls between 16 to 22.9°C., the coldest part of the year, and that the aragonite percentage of the worm tubes grown somewhere within this range is the second lowest analyzed, forms a strong argument in favor of the interpretation offered. For the balance of samples only the temperatures of the collecting dates are available. Secured over a period of rising spring and summer temperatures increasing aragonite ratios should be in evidence when grouping the samples in the order of successively higher temperatures of their collecting dates. Admittedly this constitutes a rather crude approximation, particularly since the tubes at the time of collecting had not been examined to see whether they were alive, that is, were growing in the immediately preceding time-temperature interval. As shown in table 2, there is in their successive scatter ranges a gross in-

TABLE 2

LOCATION	T. °C. AT TIME OF COLLECTING	ARAGONITE, % MIN.	MAX.	MEAN
Harrington Sound	25	58	77	67
Cock Rocks	27	68	80	75
Emily Bay	27	66	96	77
Harrington Sound	29	80	89	85

crease in aragonite percentages corresponding to increasing temperatures of the date of collection, minimum values and range means in particular exhibiting definite alignment. The deviation in the two sample series taken at the same temperature from Emily Bay, an inshore bay, and of Cock Rocks, facing the open ocean on the south side of the islands, could well be attributed in part to their respective microenvironmental climates and to differences in cumulative growth periods of the tubes analyzed. It would thus seem that within the limits of the means of comparison the Bermuda sample series indicates that temperature control of the skeletal modification composition in this biotic group extends clear down to the local population level, hence in principle corroborates the gross trend relations. The three sets of samples from Gloucester, Virginia, show a lesser degree of correlation than the Bermuda ones (figure 1), despite the fact that they were derived from test stakes for which the length of submergence and corresponding temperature and salinity data were made available by Dr. Sheltema. The aragonite percentages of the two extremes in temperature range derivation, as far as the period of submergence of their respective substrates is concerned, follow the generally indicated trend, but the intermediate one very close to the highest temperature derived sample

does not. Of the possible reasons to account for the phenomena, partial growth cover of the submergence period of the stake seems reasonable, as the worm tubes here were considerably smaller and more slender than those from the two other ones. If so, their growth period had to be confined to temperatures in the low twenties. In the absence of information on whether the *Hydroides* individuals were alive at the time of the stake recovery this interpretation cannot be verified.

Reference has been made earlier to deviations from the growth trend which decidedly fall far beyond the limits of error of the method involved. Specifically these pertain to the single sample from Malmøya in Oslo fjord, Norway, and the Bermuda samples from Mangrove Lake. The aspect in common to both is that their respective aragonite percentages lie above the upper limits suggested by the scatter band width in the gross trend relations inferred. In both cases the samples were derived from environments where the salinity is either low, as is the case of Malmøya, or in the case of Mangrove Lake markedly fluctuated over a considerable range during the year, a point in common only with the samples of Gloucester, Virginia, with ranges extending down to as low as 18 per mille. By contrast the salinities of the habitats of all other samples lie above 30 per mille and fluctuate only over a narrow range if at all. The inference might be drawn that salinity may in these two instances account for the deviations, and if so would indicate that low salinities tend to raise the aragonite synthesis above that determined by environmental temperatures alone. However, there is no obvious correlation in the three Gloucester samples. As far as the incidence of an observed temperature effect is concerned, a preliminary survey of other carbonate secreting organisms has shown that it extends outside of the serpulidae to the spirorbidae among the polychaete worms, and also embraces the genera *Modiolus*, *Mytilus*, *Pedalion*, *Pteria*, *Pinctada*, *Pinna*, *Pecten*, *Lima*, *Anomia* among the pelecypods, and the genera *Patella*, *Haliotis*, *Fissurella*, *Nerita*, and *Littorina* among the gastropods. The mode of temperature response in the Spirorbis group seems to differ from that in the case of the serpulidae in that the tubes from mean environmental temperatures of 18°C. upward are entirely composed of aragonite, and hence seem to show responses in modification composition only in the lower temperature ranges.

In the mollusca the mineralogic composition and their temperature responses, where observed, differ markedly from genus to genus and also within genera from species to species. An exploratory study of this relationship in the mollusca cited permits evaluation of certain aspects which could not be presented in the case of the serpulidae—for example, the variations of the modification compositions within the population of a single species. In the case of the serpulidae the entire yearly temperature range has to be considered, since members of individuals which could have grown at any time had to be used for single analyses. In the case of *Mytilus edulis* from Ocean City, Maryland, presented hereafter, the analyses were made on individuals grown over a short period, within a smaller specified temperature range. The specimens were derived from test stakes submerged over a period of 30 days during which the temperature increased from 17.1 to 23.2°C. Selecting a growth series of seven individuals representative of the entire size range, the analyses revealed a range in aragonite ratios for the shells from 35 to 69%. Assuming the weight of a given shell to correspond to age, with due regard given to differential weight increases in age selected population segments, and plotting the weights of

individual shells against their respective aragonite percentages, a trend of progressive decrease in percentages with increasing weight is observed (Fig. 2). Since larger shells imply correspondingly longer growth periods and thus include more of the colder initial temperatures characteristic of the earlier part of the 30-day period, the larger shells should then include more calcite, and thus have a lower aragonite-calcite ratio. Thus the inference of the mineralogic-temperature relationship shown by the Serpulidae is confirmed by the data for the *Mytilus* example. Further if the mean temperatures of the localities for the Serpulidae are plotted against the mean aragonite content of all samples of a given locality, the curve shown in figure 1 is obtained. Since it is impossible to specify the correct temperature ranges for any one sample, and since it is possible that the serpulidae in a specific locality, such as Bermuda, will grow throughout the year, a plot of the mean mineralogic compositions against the mean temperatures should convey a truer relationship. It is

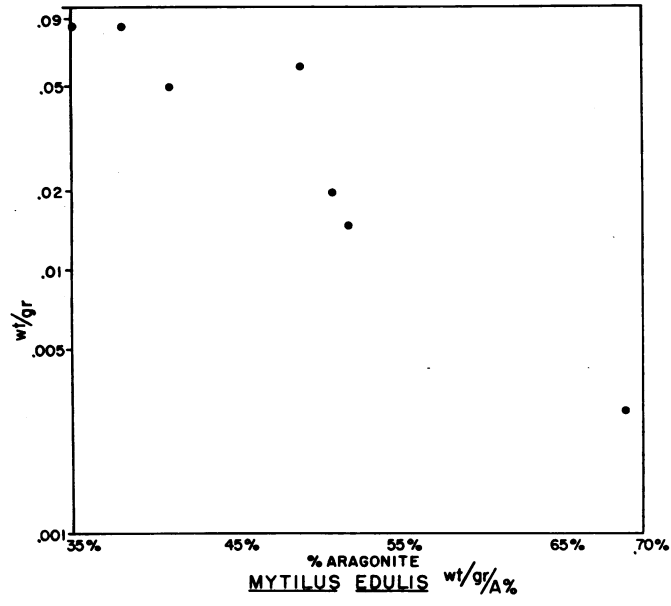

FIGURE 2

perhaps worth noting that the point most divergent from a linear relationship includes only one analysis (Malmøya) and thus could embody a growth period of large uncertainty as far as the temperature range is concerned, which in this case is from 0.22–23.03°C.

Another aspect which had not been considered in the analyses of the serpulidae data pertains to the comparative secretion behavior in modification synthesis products of various species. The existence of a species effect is demonstrated by the Mytilidae. *Smaragdensis* from the China Coast and the Philippines and *perna* from Ilha Grande, Brazil, occupy the tropical temperature niche and were composed of 100% aragonite. If there were no species effect, if the above species behaved in their modification synthesis analogous to *Mytilus edulis*, previously discussed, then the Brazilian species, *perna*, living in a locality with low enough temperatures during

part of the year (18.9–27.8°C.) to overlap with those of *edulis* at Ocean City, Maryland (17.1–23.2°C.) should contain some calcite in its skeletal makeup. Since the limited data indicate this is not true a species differentiation in responses is inferred. Investigations of the cross-sectional temperature records of some recent mollusc shells by means of the O^{18}/O^{16} ratio have shown that the temperature niche of shell deposition for a given species may not coincide with its temperature tolerance. In *Chama macerophilla*, an extreme case, shell deposition at Bermuda was shown to occur only at the elevated temperatures.[12] The species-determined differences in modification depositional responses noted between *Mytilus edulis* and *Mytilus perna* may perhaps be attributed to a similar phenomenon, offsetting the temperature threshold limits of their respective shell secretionary ranges, in that *perna* does not lay down shell in the overlapping temperatures, that is, below 23°C. This distinct possibility points to one of the possible controls of the observed differences in species-determined modification synthesis products.

However, in this particular example the species do not occupy overlapping geographic ranges. An example illustrating the most extreme differentiation in two gastropod species, occupying in overlapping depth ranges the inter-tidal zone on the Pacific coast, is *Littorina scutulata* and *L. planaxis*. Populations of the two species secured alive *in situ* at the same locality at La Jolla, California, analyzed 100% aragonite for *planaxis*, while those of *scutulata* are composed of both calcite and aragonite, with aragonite ratios ranging from 21 to 45%. Thus we have an example of two species essentially occupying the same environmental niche, one being environment-independent as far as its ecologic valence with reference to the local climatic conditions, the other temperature-dependent in its modification synthesis products.

While a temperature-modification compositional selection has been shown to exist in the groups under consideration, the possibility of other less marked ecologic effects superimposed on the former cannot be ignored. Their evaluation, if they exist, is not possible with the data at hand. It seems tempting, as indicated above, to interpret those deviations from the gross trend in the Serpulidae and Mytilidae with apparently high aragonite percentages from the Baltic Sea, as the result of other effects, in particular of lowered or fluctuating salinities superimposed on the temperature effect.

The data presented call clearly for an amending of the generalization that biochemical carbonate synthesis in skeletal form with reference to modification composition is genetically fixed, at least at the species level. While this appears to be the case in most of the phyla of carbonate secreting forms, evidence has been presented to show that exceptions do exist. These involve at present components of representatives within two phyla of higher levels of organization, the serpulidae and spirorbidae among the polychaete worms, and numerous genera of certain orders within the two molluscan classes gastropods and pelecypods. Within these the skeletal modification composition is dependent in numerous species upon factors of the environmental framework, definitely upon temperature. The temperature effect is expressed by the increase in aragonite over calcite with elevation in temperatures. Species-determined responses, differing in the modes of behavior in modification synthesis, are shown to be effective in the molluscan examples. Hence there is evidence that ecology, in the form of temperature, directly or indirectly

influences biochemical carbonate secretion in the skeletal modification synthesis by governing its composition, which is also potentially subject to the modifying effects of species controls.

One obvious implication of this modified concept of environmentally-affected aragonite ratios concerns the chemistry of the major trace element strontium in skeletal carbonate. Noll[13] has shown that the lattice uptake of this element is governed by the modification type of the carbonate involved. It is comparatively small, up to 0.14% in the stable modification calcite; in the metastable phase aragonite it can be maximally 4.69%, calculated as SrO.[13] In skeletal carbonates Odum[14] and Kulp, *et al*,[15] to cite the most recent references, have stated that concentration levels of strontium differ not only according to the carbonate modifications involved, but also as a function of strontium concentration in the aqueous medium, and are sensitive to biologic differentiation within the same mineralogic modification. However, as far as other environmental factors are concerned, they imply that the strontium content is independent of temperature. Partially correct in principle the statement requires revision as far as its generalization is concerned. Within the groups shown here to be at least temperature-dependent an increase in strontium concentrations should be in evidence as the result of the increase in aragonite. Figure 3 shows the relation of strontium concentration, calculated as strontium carbonate, in the serpulidae worm tubes to corresponding aragonite percentages. An increase in strontium carbonate with increase in the aragonite percentages is clearly in evidence. This obviously adds another complicating factor to the many already in evidence in tracing the history of the strontium cycle through geologic time, as it involves consideration of the distinct possibility of evolutionary changes in skeletal carbonate modifications and ecologic responses, two aspects intriguing in themselves from the biologic point of view.

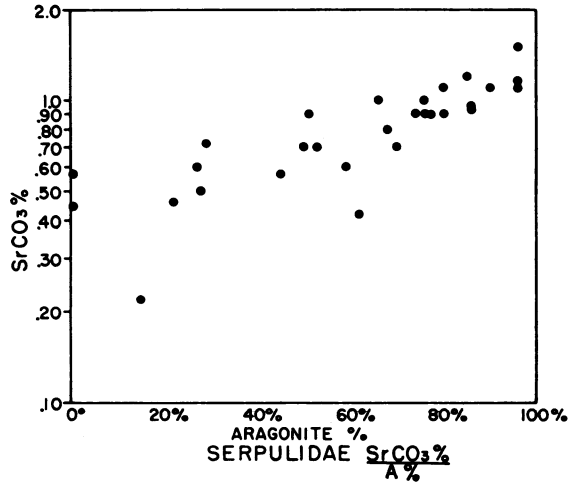

FIGURE 3

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